

## Research Article

**Microwave heating induces changes in the physicochemical properties of baru (*Dipteryx alata* Vog.) and soybean crude oils****Thays Helena Borges<sup>1</sup>, Ricardo Malheiro<sup>2, 3</sup>, Adriana Marques de Souza<sup>1</sup>, Susana Casal<sup>3</sup> and José Alberto Pereira<sup>2</sup>**<sup>1</sup> School of Agronomy and Food Engineering, Federal University of Goiás, Campus Samambaia – Rodovia Goiânia/Nova Veneza, Goiânia, Brazil<sup>2</sup> Mountain Research Centre (CIMO)/School of Agriculture, Polytechnic Institute of Bragança, Campus de Sta Apolónia, Bragança, Portugal<sup>3</sup> REQUIMTE/Laboratório de Bromatologia e Hidrologia, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal

Baru oil is extracted from baru nuts (*Dipteryx alata* Vog.) by cold mechanical pressing, and is exploited as a source of vitamins, fatty acids and antioxidants in the Brazilian food and pharmaceutical sectors. No information is available on this oil under domestic culinary processes and thermal conditions. So, in the present study we evaluated the response of crude baru oil under microwave heating (0, 1, 3, 5, 10, and 15 min), using crude soybean oil as comparison. Physical and chemical parameters were evaluated (free acidity, peroxide value, specific extinction coefficient at 232 and 270 nm,  $\Delta K$  and color by CIELAB method), fatty acid profile, tocopherol composition, antioxidant activity, and oxidative stability. Until 3 min (1000 W) no significant adverse changes were observed in either oil. However, higher exposition times are more adverse to baru oil than to soybean oil. Tocopherols, oils stability and antioxidant activity drop abruptly. The typical yellow coloration is lost with heating, giving a less appealing appearance to the oils. By a principal component analysis, it was verified that microwave heating differently influenced each oil, and within the same oil, exposure time also caused distinct effect on properties, quality, and composition. Based on the obtained results, we discourage the use of baru oil for culinary process.

**Practical applications:** The use of baru oil for prolonged culinary processes is discouraged due to lower stability and low content in antioxidants. Baru oil is more suitable for seasoning for usage in domestic consumption at RT. Exposure to microwave heating is completely discouraged at an exposure higher than 3 min.

**Keywords:** Crude baru oil / *Dipteryx alata* Vog. / Microwave heating / Oil composition / Oil quality

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## 1 Introduction

Brazil is a country rich in biodiversity, and has many vegetable species, which are known to produce oils with unique aromas

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**Abbreviations:** DPPH, 2,2-diphenyl-1-picrylhydrazyl;  $\Delta E$ , color differences; FA, free acidity; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; PCA, principal component analysis; PV, peroxide value; SFA, saturated fatty acids; YI, yellowness index

and flavors. Properties of these vegetable oils have been extensively researched and exploited, mainly by international companies, due to their various applications in food, pharmaceutical, cosmetic, and other industrial sectors [1]. Baru oil is extracted from fruit almonds of baru (*Dipteryx alata* Vog.), growing almost exclusively in the Brazilian Cerrado. The oil is obtained exclusively by mechanical processes, namely a cold pressing extraction method. The crude oil obtained is not submitted to refinement, being commercialized on its crude form. While there is some recent knowledge about baru almonds characterization, composition and properties [2–5], knowledge about crude baru oil is practically inexistent. The few reports on the characterization of the oils from baru almonds respects to oils extracted with organic

solvents [6–9], which does not correspond to the normally consumed baru oil. Nevertheless, baru oil is commercialized as being a rich source of antioxidants with beneficial effects to consumers' health, as well as a pool of vitamins and  $\omega$ -6 and  $\omega$ -9 fatty acids. Baru oil is used in the Brazilian cuisine, in diversified dishes, being used raw to flavor salads, or as an ingredient in diverse cooking processes. However, the performance and stability of this kind of vegetable oil under culinary and thermal process is not reported as well. Such information is needed to understand its stability under adverse thermal conditions, and to observe its degradation pattern.

The modern way of life obliges individuals to reduce the time dedicated to prepare meals to the minimum possible. Under such circumstances, microwave oven is increasingly an indispensable utensil in modern kitchens. These equipment's have the capacity to rapidly transmit heat, due to microwaves high penetration power, are easy to use, and allow to reduce time, effort and energy comparatively to conventional culinary methods [10]. Meanwhile, microwave oven applications showed to be harmful to vegetable oils, being possible to observe their deterioration in few minutes. Several reports highlight vegetable oils quality degradation, reduction in bioactive compounds and properties, fatty acids degradation, pigments destruction, sensorial changes, and color modifications, as well as physical and rheological changes [11–20]. In fact, comparatively to a conventional oven heating, microwave heating induces higher and faster oils deterioration [21–23].

In this sense, with the present work we intend to investigate the response of crude baru oil, taking crude soybean oil as comparison due to its widespread consumption worldwide, under microwave heating conditions at different exposure times. Changes in the quality (free acidity, peroxide value,  $K_{232}$ ,  $K_{270}$ ,  $\Delta K$  values), color, fatty acids profile, tocopherols composition, antioxidant activity, and oxidative stability were evaluated.

## 2 Materials and methods

### 2.1 Sampling of vegetable oils

To assess the behavior of crude baru and soybean oils under microwave heating conditions, commercial samples were produced in Brazil and obtained from local markets (3 bottles of 1 L per oil). All oils were 1 year inside the expiration date. From each oil, each bottle was considered an independent sample ( $n = 3$ ), with three measurements per sample (unless otherwise stated) and per parameter studied.

### 2.2 Heating procedure

To simulate conventional times used in microwave domestic cooking, different exposure times were tested, namely, 1, 3, 5,

10, and 15 min. Within each oil studied one sample, in triplicate, was used as control sample (unheated –  $t_{0min}$ ). From each subsample, approximately 45 g (0.9 cm of thick layer) of oil were individually heated in Petri dishes (20 mm high and 110 mm of diameter) in a domestic microwave oven of 20 L (AMSTRAD) at maximum power (1000 W). For each vegetable oil, for each exposure time tested the Petri dishes (95 cm<sup>2</sup> of exposure area with air) were heated individually in the microwave heating with constant rotation. The cold samples were transferred to Falcon tubes and kept under refrigeration until analysis.

### 2.3 Quality parameters determination

Free acidity (expressed as percentage of oleic acid), peroxide value (expressed as mEq. O<sub>2</sub>/kg of oil) and coefficients of specific extinction at 232 and 270 nm ( $K_{232}$  and  $K_{270}$ ) were determined according to European Union standard methods [24] (Annexes II and IX in European Community Regulation EEC/2568/91 from 11th July).

### 2.4 Color determination

Color of the vegetable oils was determined with a Konica Minolta model CR-400 colorimeter. Color differences ( $\Delta E$ ) between control samples considered as standard (unheated oils) and oils heated at different exposure times were calculated from the determined monochromatic variables  $L^*$ ,  $a^*$ , and  $b^*$  obtained from CIELAB method, as well as yellowness index (YI) as described by Zamora *et al.* [25]:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

$$YI = \frac{142.86 \times b^*}{L^*}$$

$L^*$  is a measure of luminance or lightness component, which ranges from 0 to 100 (black to white);  $a^*$  ranges from negative to positive (green to red); and  $b^*$  also ranges from negative to positive (blue to yellow).

### 2.5 Fatty acid composition

Fatty acids were evaluated as their methyl esters after cold alkaline transesterification with methanolic potassium hydroxide solution [24] and extraction with *n*-heptane. The fatty acid profile was determined with a Chrompack CP 9001 chromatograph equipped with a split-splitless injector, a FID detector, an autosampler Chrompack CP-9050 and a 50 m  $\times$  0.25 mm i.d. fused silica capillary column coated with a 0.19  $\mu$ m film of CP-Sil 88 (Varian). Helium was used as carrier gas at an internal pressure of 110 kPa. The temperatures of the detector and injector were 250 and 230°C,

respectively. The oven temperature was programmed at 120°C during the first 3 min with an increase of 4°C/min until 220°C. The split ratio was 1:50 and the injected volume was of 1 µL. The results are expressed in relative percentage of each fatty acid, calculated by internal normalization of the chromatographic peak area eluting between myristic and lignoceric methyl esters. A control sample (olive oil 47118, Supelco) and a fatty acids methyl esters standard mixture (Supelco 37 FAME Mix) was used for identification and calibration purposes (Sigma, Spain).

## 2.6 Tocopherol composition

Tocols were evaluated following the international standard ISO 9936 [26], with some modifications as described by Malheiro et al. [27]. Tocopherols standards ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ) were purchased from Calbiochem (La Jolla, San Diego, CA) and Sigma, while the internal standard 2-methyl-2-(4,8,12-trimethyltridecyl)chroman-6-ol (tocol) was from Matreya Inc. (Pleasant Gap, PA). A 80 mg amount of filtered of baru oil or 40 mg amount of filtered of soybean oil was blended with an appropriate amount of internal standard solution (tocol) in a 1.5 mL volume of *n*-hexane and homogenized by stirring. Sample preparation was conducted in dark and tubes containing the samples were always wrapped in aluminum foil. The mixture was centrifuged for 5 min at 13 000 rpm and the supernatant analyzed by HPLC. The liquid chromatograph consisted of a Jasco integrated system (Japan) equipped with a Jasco LC – NetII/ADC data unit, a PU-1580 Intelligent Pump, a LG-1580-04 Quaternary Gradient Unit, a DG-1580-54 Four Line Degasser and an FP-920 fluorescence detector ( $\lambda_{\text{exc}} = 290 \text{ nm}$  and  $\lambda_{\text{em}} = 330 \text{ nm}$ ). The chromatographic separation was achieved on a Supelcosil<sup>TM</sup> LC-SI column (3 µm) 75 mm × 3.0 mm (Supelco, Bellefonte, PA), operating at constant RT (23°C). A mixture of *n*-hexane and 1,4-dioxane (97.5:2.5 v/v) was used as eluent, at a flow rate of 0.7 mL/min. Data were analyzed with the ChromNAV Control Center – JASCO Chromatography Data Station (Japan). The compounds were identified by chromatographic comparisons with authentic standards, by co-elution and by their UV spectra. Quantification was based on the internal standard method, using the fluorescence signal response.

## 2.7 Radical scavenging activity

The samples were analyzed for their capacity to scavenge the stable DPPH radical as described by Kalantzakis et al. [28]. A 1 mL amount of oil solution in ethyl acetate (10%, w/v) was added to 4 mL of a freshly prepared DPPH radical solution ( $1 \times 10^{-4} \text{ M}$  in ethyl acetate) in a screw-capped 15 mL test tube. The reaction mixture was shaken vigorously for 10 s and the tube was maintained in the dark for 30 min. The absorbance of the mixture was measured at 515 nm against a blank solution. A control sample was prepared and measured daily.

## 2.8 Oxidative stability (Rancimat)

The oxidative stability was estimated by measuring the oxidation induction time, on a Rancimat 743 apparatus (Metrohm CH, Switzerland). Filtered, cleaned, dried air (20 L/h) was bubbled through the oil (3.00 g) heated at  $120 \pm 1.6^\circ\text{C}$ , with the volatile compounds being collected in distilled water, and the increasing water conductivity continuously measured. The time taken to reach the conductivity inflection was recorded.

## 2.9 Statistical analysis

### 2.9.1 Analysis of variance

The analysis of variance of the quality and color parameters as well as chemical composition data was analyzed by the SPSS software, version 21.0 (IBM Corporation, New York, USA). The differences in each oil during the exposition times and between oils in each exposition time were analyzed using one-way ANOVA followed by Tukey's HSD with  $\alpha = 0.05$ .

### 2.9.2 Regression analysis

A regression analysis, using Excel from Microsoft Corporation, was established between the different microwave heating exposure times and the quality parameters (free acidity, peroxide value,  $K_{232}$ ,  $K_{270}$ ,  $\Delta K$ ), oxidative stability, fatty acids profile and tocopherols content, and antioxidant activity for all the samples.

### 2.9.3 Principal components analysis (PCA)

Principal components analysis (PCA) was applied for reducing the number of variables (17 variables corresponding to the quality parameters – free acidity, peroxide value,  $K_{232}$ ,  $K_{270}$ , and  $\Delta K$ ; radical scavenging activity of DPPH, oxidative stability,  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherol, total tocopherols content; and monochromatic variables obtained by CIELAB ( $L^*$ ,  $a^*$ , and  $b^*$ ), YI and  $\Delta E$ ) to a smaller number of new derived variables (principal component or factors) that adequately summarize the original information, i.e., the effect of microwave heating on baru and soybean crude oils. Moreover, it allowed recognizing patterns in the data by plotting them in a multidimensional space, using the new derived variables as dimensions (factor scores). PCA was performed by using SPSS software, version 21.0 (IBM Corporation).

## 3 Results and discussion

### 3.1 Effect of microwave heating on vegetable oil quality parameters

Free acidity (FA), peroxide value (PV), and specific extinction coefficients ( $K_{232}$  and  $K_{270}$ ) were determined in

**Table 1.** Effect of microwave heating in free acidity (%), peroxide value (mEq. O<sub>2</sub>/kg), specific extinction coefficients ( $K_{232}$ ,  $K_{270}$ , and  $\Delta K$ ) in crude baru and soybean oils (mean  $\pm$  SD;  $n = 3$ )

	$t_{0\text{min}}$	$t_{1\text{min}}$	$t_{3\text{min}}$	$t_{5\text{min}}$	$t_{10\text{min}}$	$t_{15\text{min}}$	$R^2$	$P$
Free acidity								
Baru oil	0.28 $\pm$ 0.04 Aa	0.35 $\pm$ 0.04 Ab	0.37 $\pm$ 0.03 Ab,c	0.38 $\pm$ 0.04 Ab,c	0.39 $\pm$ 0.04 Ab,c	0.41 $\pm$ 0.03 Ac	0.764	***
Soybean oil	0.75 $\pm$ 0.04 Ba	0.70 $\pm$ 0.00 Ba	0.72 $\pm$ 0.04 Ba	0.73 $\pm$ 0.04 Ba	0.72 $\pm$ 0.06 Ba	0.75 $\pm$ 0.05 Ba	0.015	n.s.
Peroxide value								
Baru oil	2.4 $\pm$ 0.3 Aa	2.6 $\pm$ 0.3 Aa	2.78 $\pm$ 0.4 Aa	8.0 $\pm$ 0.4 Ab	21.5 $\pm$ 1.3 Ac	23.6 $\pm$ 1.2 Ad	0.825	***
Soybean oil	3.5 $\pm$ 0.5 Ba	4.3 $\pm$ 0.5 Ba	3.9 $\pm$ 0.4 Ba	5.7 $\pm$ 0.7 Bb	15.9 $\pm$ 1.3 Bc	17.6 $\pm$ 1.3 Bd	0.779	***
$K_{232}$								
Baru oil	2.02 $\pm$ 0.23 Aa	2.23 $\pm$ 0.08 Aa	2.42 $\pm$ 0.15 Aa	3.05 $\pm$ 0.17 Ab	5.77 $\pm$ 0.60 Ac	7.62 $\pm$ 0.36 Ad	0.821	***
Soybean oil	3.63 $\pm$ 0.38 Ba	4.02 $\pm$ 0.49 Ba	4.20 $\pm$ 0.20 Ba	4.32 $\pm$ 0.65 Ba	8.96 $\pm$ 0.81 Bb	8.93 $\pm$ 1.01 Bb	0.925	***
$K_{270}$								
Baru oil	0.42 $\pm$ 0.03 Aa	0.44 $\pm$ 0.02 Aa	0.44 $\pm$ 0.04 Aa	0.55 $\pm$ 0.06 Ab	0.93 $\pm$ 0.08 Ac	1.08 $\pm$ 0.04 Ad	0.809	***
Soybean oil	0.42 $\pm$ 0.01 Aa	0.47 $\pm$ 0.05 Aa	0.63 $\pm$ 0.06 Ba	1.02 $\pm$ 0.14 Bb	1.33 $\pm$ 0.08 Bc	1.88 $\pm$ 0.31 Bd	0.729	***
$\Delta K$								
Baru oil	0.022 $\pm$ 0.001 Aa,b	0.021 $\pm$ 0.001 Aa,b	0.020 $\pm$ 0.003 Aa	0.030 $\pm$ 0.004 Ab	0.049 $\pm$ 0.013 Ac	0.072 $\pm$ 0.009 Ad	0.770	***
Soybean oil	0.027 $\pm$ 0.001 Ba	0.032 $\pm$ 0.004 Ba	0.037 $\pm$ 0.006 Ba	0.063 $\pm$ 0.011 Bb	0.104 $\pm$ 0.001 Bc	0.099 $\pm$ 0.017 Bc	0.875	***

Means within a line, in each vegetable oil and each parameter studied, with different minor letters differ significantly ( $P < 0.05$ ); in each column, among vegetable oils, for each microwave heating exposure time and parameter studied, mean values with different capital letters differ significantly ( $P < 0.05$ ).

$P > 0.05$ , n.s., not significant correlation;  $*P \leq 0.05$ , significant correlation;  $**P \leq 0.01$ , very significant correlation;  $***P \leq 0.001$ , extremely significant correlation.

order to assess the effect of microwave heating in baru and soybean crude oils quality, being such results reported in Table 1. Free acidity is an analytical parameter frequently used to evaluate the hydrolysis extension in vegetable oils during thermal process. An increase in this parameter indicates a higher presence of free fatty acids in the vegetable oil, a direct consequence of hydrolysis, being an important indicator of oil chemical deterioration. Unheated baru oils reported lower FA than soybean oils (0.28% and 0.75%, respectively). However, with the increasing heating exposition times, a different response was given by each oil. Soybean oil was not affected by the increasing exposition time, even at  $t_{15\text{min}}$  (Table 1). In baru oils, a significant increase of FA was recorded after 1 min heating, comparatively with unheated oils ( $P < 0.001$ ). Thereafter, the FA increased steady and continuously with the heating time, reaching 0.41% at  $t_{15\text{min}}$ . In fact, baru oils FA values were extremely correlated with the exposure heating time, while for soybean oils no correlation was observed (Table 1). Both vegetable oils used in this study were crude oils, which explain the high FA values found comparatively to other vegetable oils. Refined vegetable oils present lower FA since during refining steps the free fatty acids are removed by neutralization [29], a fact not shared by crude oils. The results observed in soybean oils are in accordance with previous works developed by our research group, where no significant effects on FA were found after microwave heating [10, 18, 19, 30]. The different patterns observed during heating of the vegetable oils studied may be related with their extraction process. Soybean oil chemical

extraction is a more industrialized and settled technology than the one used for baru oil extraction, removing residual water more efficiently. Baru oils are extracted by cold extruding mechanism, without subsequent water removal, which might favor fatty acids hydrolysis from triglycerides and diglycerides under thermal processing.

Oxidation is another chemical process that induces degradation in vegetable oils during thermal process. The peroxide value (PV) and coefficients of specific extinction at 232 and 270 nm ( $K_{232}$  and  $K_{270}$ , respectively) are methods that allow assessing oxidation estimation and extension. During vegetable oils oxidation hydroperoxides are mainly formed from unsaturated fatty acids, being described as the primary compounds of oxidation and are determined by PV, side by side with the formation of conjugated dienic bonds systems, absorbing in the UV range of 235 nm and evaluated by the  $K_{232}$  [31]. Peroxides are unstable compounds, particularly under thermal condition, leading to the formation of secondary products of oxidation, like aldehydes, alcohols, ketones, acids, dimmers, trimmers, polymers, and cyclic compounds [32]. The extension of such secondary oxidation can be monitored at 270 nm, determining  $K_{270}$  [24], giving us the indication of extensive oxidative stress and in our case high thermal stress.  $\Delta K$  is an important parameter when oxidation studies are carried out in vegetable oils since it aids to evaluate the degree of degradation. This parameter is correlated with the state of oxidation by detecting specific oxidized compounds, some generated from secondary oxidation.

Unheated oils reported low PV, with 2.4 and 3.5 meq O<sub>2</sub>/kg in baru and soybean oils respectively (Table 1). During heating exposure no significant changes were observed in both oils until 3 min. After that PV increased significantly in both oils ( $P < 0.001$ ). After 15 min heating, baru oil increased its PV 8 times (21.5 meq O<sub>2</sub>/kg) comparatively to the initial value, while soybean oil increased only around 4.5 times (15.9 meq O<sub>2</sub>/kg). Such results show that baru oil is more labile and unstable under thermal process, increasing oxidation rate and producing higher quantities of peroxides. This observation was also confirmed with the results obtained in  $K_{232}$  values (Table 1). Soybean oil  $K_{232}$  values were higher at all exposure times but the increase was comparatively lower (2.5 times) than the one observed with baru oil (4 times) with  $t_{15\text{min}}$ . In fact, a significant increase of primary compounds of oxidation was observed sooner in baru oil ( $t_{3\text{min}}$ ), while soybean oils resisted quite longer, until  $t_{5\text{min}}$ . Concerning the formation of secondary products of oxidation, measured here by the  $K_{270}$ , soybean oils were more affected than baru oils, reflecting a higher extension of secondary oxidation. Such results are probably associated with the higher unsaturation degree of soybean oil, as will be discussed later, making them more prone to the development of conjugated triene bonds, evaluated at 270 nm.  $\Delta K$  values, reflecting the absorbance variation in the 268–270 nm region were also greater in soybean oils, mainly due to the extensive secondary oxidation that this vegetable oil suffered, as verified in  $K_{270}$  values.

All four parameters (PV,  $K_{232}$ ,  $K_{270}$  and  $\Delta K$ ), indicative of oxidation process, were positively and extremely correlated with the exposure time in both oils studied (respective  $P$ -values and  $R^2$  values reported in Table 1). This means that higher exposition time induced higher vegetable oils degradation. For soybean oil, such inference was also observed in recent works that submitted refined soybean oils to microwave heating [19, 30].

### 3.2 Effect of microwave heating on vegetable oil color

Color is an important physical parameter of food products for two main reasons: its relationship with other chemical and physical properties of food (which may be also related to processing techniques, storage conditions, and others), and its strong influence in consumers' preferences, which influence subsequent buying/consuming decisions. According to Salmerón et al. [33] color specification is often a key part of a complete food-quality control.

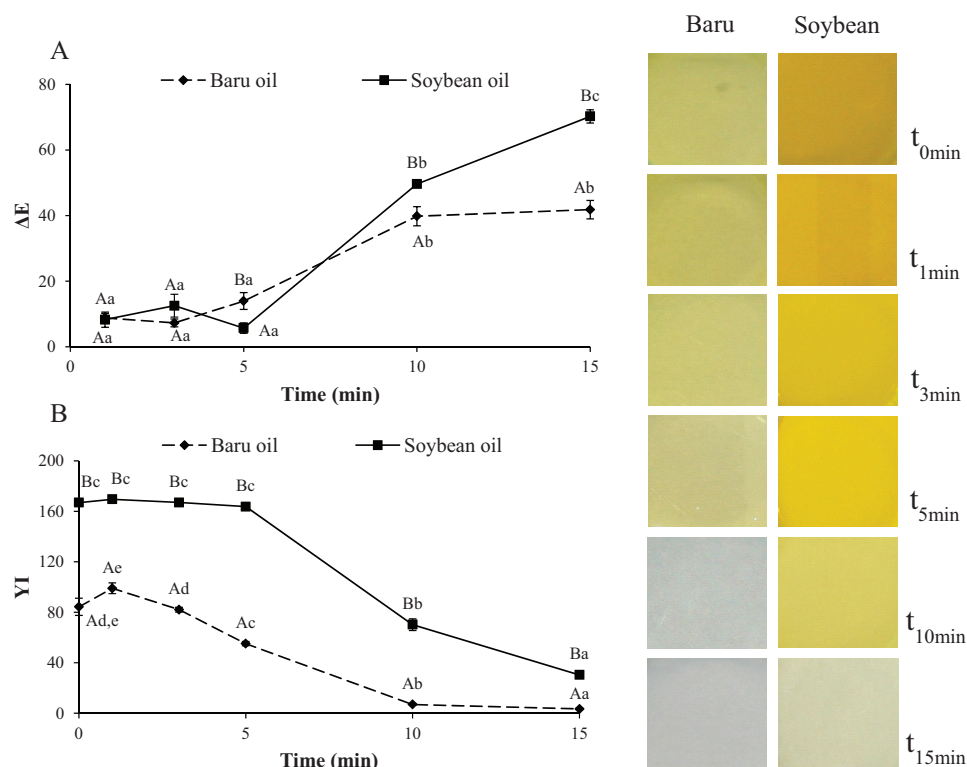
CIELAB main axes,  $L^*$ ,  $a^*$ , and  $b^*$  values obtained from both vegetable oils during microwave heating are reported in Table 2. In both vegetable oils,  $L^*$  values decreased slightly in the first minutes of heating, associated with a slight darker color, being then observed a continuous and steady increase until  $t_{15\text{min}}$ , indicative of higher luminance and a general loss of color. In general,  $a^*$  values decrease with the increasing exposition time, being observed a loss of green coloration in both vegetable oils. Baru oils presented higher greenish coloration than soybean oil in the first minutes of microwave heating, but with higher exposition times the reverse was observed. Concerning  $b^*$  values, which in vegetable oils is indicative of yellow coloration, their values decreased significantly with exposition time, with lower values in baru oil comparatively to soybean oil (Table 2). The real coloration of baru and soybean oils at the different exposure times tested is visible in Fig. 1, together with the color differences ( $\Delta E$  – Fig. 1A) and yellowness index (YI – Fig. 1B). In  $\Delta E$  is possible to observe that baru oil color changes were not significantly until  $t_{3\text{min}}$ , while in soybean oil it was prolonged up to 5 min. With higher exposition times, the color differences increased significantly, with higher differences observed in soybean oils than in baru oils. In which respects to the yellowness index (YI), soybean oil reported initially a much more intense yellow coloration (Fig. 1B). In both oils the exposure time influenced this index, with a gradual loss as the heating time

**Table 2.** Effect of microwave heating in the color parameters of baru and soybean crude oils evaluated by CIELAB method (mean  $\pm$  SD;  $n=3$ )

	$t_{0\text{min}}$	$t_{1\text{min}}$	$t_{3\text{min}}$	$t_{5\text{min}}$	$t_{10\text{min}}$	$t_{15\text{min}}$	$R^2$	$P$
Baru oil								
$a^*$	$-12.95 \pm 1.12$ Aa	$-13.51 \pm 0.41$ Aa	$-13.36 \pm 0.34$ Aa	$-10.56 \pm 0.45$ Ab	$-3.11 \pm 0.36$ Ac	$-2.32 \pm 0.33$ Ac	0.789	***
$b^*$	$46.86 \pm 5.42$ Ad	$45.77 \pm 4.04$ Ac,d	$41.48 \pm 2.42$ Ac	$28.85 \pm 2.61$ Ab	$3.66 \pm 0.94$ Aa	$1.80 \pm 0.76$ Aa	0.806	***
$L^*$	$71.76 \pm 2.98$ Aa	$70.19 \pm 1.94$ Aa	$72.22 \pm 0.77$ Aa,b	$74.18 \pm 1.12$ Ab,c	$75.23 \pm 0.88$ Ac	$75.34 \pm 0.98$ Ac	0.564	***
Soybean oil								
$a^*$	$-11.25 \pm 1.18$ Ba	$-6.74 \pm 0.45$ Bb	$-6.12 \pm 0.86$ Bb	$-11.21 \pm 1.47$ Aa	$-10.87 \pm 0.70$ Ba	$-5.46 \pm 0.18$ Bb	0.025	n.s
$b^*$	$86.10 \pm 3.89$ Bd	$82.92 \pm 1.79$ Bc,d	$80.87 \pm 4.04$ Bc	$85.08 \pm 1.18$ Bd	$36.62 \pm 3.98$ Bb	$16.26 \pm 1.25$ Ba	0.720	***
$L^*$	$73.75 \pm 4.30$ Ab	$69.86 \pm 1.28$ Aa	$69.18 \pm 3.42$ Ba	$74.24 \pm 1.59$ Ab	$74.44 \pm 1.44$ Ab	$76.42 \pm 1.19$ Ab	0.212	n.s

Means within a line, in each vegetable oil and in each parameter studied, with different minor letters differ significantly ( $P < 0.05$ ); among vegetable oils, for each microwave heating exposure time and parameter studied, mean values with different capital letters differ significantly ( $P < 0.05$ ).

$P > 0.05$ , n.s., not significant; \* $P \leq 0.05$ , significant correlation; \*\* $P \leq 0.01$ , very significant correlation; \*\*\* $P \leq 0.001$ , extremely significant correlation.



**Figure 1.** Changes inflicted by microwave heating in the color of crude baru and soybean oils, as well as color differences between unheated and heated samples (1A) and yellowness index (YI) (1B). (mean  $\pm$  SD;  $n=3$ ; in each parameter and each oil, bars with different minor letters differ significantly –  $P<0.05$ ; in each parameter, among oils in each exposure time, mean values with different capital letters differ significantly –  $P<0.05$ ).

increased. In fact,  $L^*$ ,  $a^*$ , and  $b^*$  values in baru oil were extremely correlated with the exposure heating time, while in soybean oil only  $b^*$  was correlated. Such loss in coloration is related with the thermolabile pigments present in the vegetable oils, mainly carotenoids. Such vegetable oils components, present in high amounts due to the absence of refining, are severely affected by microwave heating, being their content directly dependent on the exposure time as witnessed by Malheiro *et al.* [10] El-Abassy *et al.* [16].

### 3.3 Changes in fatty acid profile

Fatty acids are the main constituents of the saponifiable fraction of vegetable oils. Despite being evaluated for all sampling times, only the fatty acids profile at  $t_{0min}$  and  $t_{15min}$  is reported for both oils in Table 3. Baru oil fatty acids profile was dominant in oleic acid ( $C_{18:1c}$ ), with levels exceeding 45%, followed by linoleic acid ( $C_{18:2}$ ) with near 29%, and palmitic acid ( $C_{16:0}$ ) containing approximately 6.5%. The linolenic acid amounts were reduced (0.2%). This profile is similar to those reported by several authors that studied oil and fruits of *Dipteryx alata* [6–9]. Soybean oil fatty acids

profile is in accordance with the literature [19, 30], and with legal requirements [34]. Soybean fatty acids profile is rich in  $C_{18:2}$  with around 53%, follow by oleic and palmitic fatty acids (near 22.5% and 10.5%, respectively), with nearly 7% of linolenic acid. Comparing the fatty acids profile of both vegetable oils it is inferred that baru oil is predominantly monounsaturated while soybean oil is mainly polyunsaturated (Table 3). Still, microwave heating inflicted changes in fatty acids profile of both oils. Analyzing the fatty acids by their common nature, grouped according to their unsaturation degree of the hydrocarbon chain, the most affected fraction was the PUFA, directly related with their higher number of double bonds, with higher susceptibility to oxidation [35, 36]. With  $t_{15min}$  of heating, PUFA content decreased around 1% in both oils, from 29.2% to 28.1% in baru oil and from 61.2% to 60.0% in soybean oil (Table 3). This happens mostly at expenses of the degradation of linoleic and linolenic ( $C_{18:3}$ ) fatty acids. In baru oil linoleic acid was more affected, due to the low amounts of linolenic acid, but in soybean oils both linoleic and linolenic acids were affected in a similar extent. The same behavior was checked in commercial refined soybean oils submitted to microwave

**Table 3.** Fatty acid profile (%) of baru and soybean crude oils, before heating ( $t_{0\text{min}}$ ) and at  $t_{15\text{min}}$  of microwave heating (mean  $\pm$  SD;  $n = 3$ )

Fatty acid	Baru oil		Soybean oil	
	$t_{0\text{min}}$	$t_{15\text{min}}$	$t_{0\text{min}}$	$t_{15\text{min}}$
C <sub>14:0</sub>	0.03 $\pm$ 0.00	0.03 $\pm$ 0.00	0.09 $\pm$ 0.02	0.08 $\pm$ 0.01
C <sub>16:0</sub>	6.32 $\pm$ 0.12	6.57 $\pm$ 0.03	10.57 $\pm$ 0.01	10.78 $\pm$ 0.12
C <sub>16:1</sub>	0.08 $\pm$ 0.01	0.09 $\pm$ 0.02	0.23 $\pm$ 0.00	0.24 $\pm$ 0.02
C <sub>17:0</sub>	0.08 $\pm$ 0.00	0.09 $\pm$ 0.00	0.14 $\pm$ 0.00	0.15 $\pm$ 0.01
C <sub>17:1</sub>	n.d.	n.d.	0.05 $\pm$ 0.01	0.04 $\pm$ 0.00
C <sub>18:0</sub>	4.88 $\pm$ 0.04	5.05 $\pm$ 0.02	3.79 $\pm$ 0.03	4.02 $\pm$ 0.00
C <sub>18:1c</sub>	45.81 $\pm$ 0.05	46.28 $\pm$ 0.04	22.45 $\pm$ 0.23	22.91 $\pm$ 0.10
C <sub>18:2cc</sub>	28.95 $\pm$ 0.02	27.94 $\pm$ 0.17	53.37 $\pm$ 0.07	52.78 $\pm$ 0.08
C <sub>18:3n6</sub>	n.d.	n.d.	0.24 $\pm$ 0.00	0.21 $\pm$ 0.00
C <sub>18:3n3</sub>	0.21 $\pm$ 0.01	0.18 $\pm$ 0.01	7.62 $\pm$ 0.00	7.05 $\pm$ 0.02
C <sub>20:0</sub>	1.26 $\pm$ 0.00	1.27 $\pm$ 0.02	0.39 $\pm$ 0.00	0.41 $\pm$ 0.00
C <sub>20:1n9</sub>	2.83 $\pm$ 0.01	2.83 $\pm$ 0.02	n.d.	n.d.
C <sub>21:0</sub>	0.06 $\pm$ 0.01	0.06 $\pm$ 0.00	0.03 $\pm$ 0.00	0.04 $\pm$ 0.01
C <sub>22:0</sub>	4.16 $\pm$ 0.08	4.19 $\pm$ 0.07	0.44 $\pm$ 0.05	0.43 $\pm$ 0.01
C <sub>22:1n9</sub>	0.36 $\pm$ 0.01	0.35 $\pm$ 0.00	0.06 $\pm$ 0.01	0.05 $\pm$ 0.00
C <sub>24:0</sub>	4.94 $\pm$ 0.11	4.94 $\pm$ 0.09	0.14 $\pm$ 0.00	0.17 $\pm$ 0.00
SFA	21.72 $\pm$ 0.01	22.19 $\pm$ 0.12	15.59 $\pm$ 0.01	16.09 $\pm$ 0.11
MUFA	49.07 $\pm$ 0.04	49.56 $\pm$ 0.04	22.81 $\pm$ 0.07	23.23 $\pm$ 0.09
PUFA	29.16 $\pm$ 0.02	28.12 $\pm$ 0.18	61.23 $\pm$ 0.07	60.04 $\pm$ 0.05
Trans	0.04 $\pm$ 0.00	0.13 $\pm$ 0.02	0.18 $\pm$ 0.00	0.25 $\pm$ 0.01

n.d., not detected.

heating at the same conditions used for this study [19, 30]. Another important observation is the influence of microwave heating in the formation of *trans* fatty acids by isomerization. Baru oil reported lower initial contents of *trans* fatty acids (0.04% vs. 0.18%). Nevertheless when exposed to  $t_{15\text{min}}$  *trans* fatty acids in both vegetable oils increased similarly (0.09% in baru oil; 0.07% in soybean oil) Such data corroborate the drastic effect of microwave heating on vegetable oils. However, the final amounts are still within the usual limits for refined oils, being therefore of no concern regarding potential health effects derived from this *trans* fatty acids formed, usually associated with the occurrence of cardiovascular disease [37, 38].

### 3.4 Tocopherol changes during microwave heating

Tocopherols are fat-soluble vitamins and natural antioxidants that have influence on the *shelf life* of vegetable oils, preserving it from rancidity by interrupting the oxidative chain reactions that culminate in the formation of hydroperoxides [39]. Among tocopherols,  $\alpha$ -tocopherol is biologically the most active one, taking also protective action in oils thermal oxidation, being consumed within this process and therefore declining with heating conditions [12].

In Table 4 is presented the tocopherols profile ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ) of baru and soybean crude oils exposed to different microwave heating times. In baru oil three tocopherols were

identified and quantified:  $\alpha$ -,  $\beta$ -, and  $\gamma$ -tocopherol, being  $\alpha$ -tocopherol the most abundant with 7.63 mg/100 g of oil. Without heating, baru oil reported a total content of tocopherols of 13.1 mg/100 g of oil, values close to those found by Takemoto et al. [7]. In soybean oil, the four forms of tocopherols were present, being  $\gamma$ -tocopherol the most abundant (62.9 mg/100 g) followed by  $\delta$ -tocopherol (33.0 mg/100 g at  $t_{0\text{min}}$ ). Total tocopherols content in soybean oil exceeds 1 mg/g of oil, nearly ten times more than that reported by baru oil. With microwave heating, for both oils studied, extremely negative significant correlations were established between tocopherols content and total tocopherols amount with the increasing exposure heating times (see respective  $P$ -values and  $R^2$  values in Table 4). Concerning total tocopherols amount, baru oil reported significant losses with very low heating times ( $t_{3\text{min}}$ ), while soybean oil only suffer significant losses at  $t_{10\text{min}}$ . With 15 min of microwave heating, baru oil reported losses of about 94.5% of its initial content, while soybean oil lost only nearly 21% of total tocopherols content. This corroborates that soybean oil, despite its increased unsaturation degree, is far more stable than baru oil under microwave processing, derived probably from its higher amount of tocopherols. According to Evans et al. [40], tocopherols have different antioxidant potential, being  $\alpha$ -tocopherol the most potent, followed by  $\gamma$ - and then  $\delta$ -tocopherol. Baru oil and soybean oil presents similar amounts of  $\alpha$ -tocopherol, but there are huge differences

**Table 4.** Effect of microwave heating in the tocopherol composition (mg/100 g) of baru and soybean crude oils (mean  $\pm$  SD;  $n = 3$ )

	$t_{0\text{min}}$	$t_{1\text{min}}$	$t_{3\text{min}}$	$t_{5\text{min}}$	$t_{10\text{min}}$	$t_{15\text{min}}$	$R^2$	$P$
<b>Baru oil</b>								
$\alpha$ -Tocopherol	7.63 $\pm$ 0.16 Ad	7.52 $\pm$ 0.01 Ad	5.65 $\pm$ 0.18 Ac	1.16 $\pm$ 0.17 Ab	0.39 $\pm$ 0.06 Aa	0.42 $\pm$ 0.00 Aa	0.755	***
$\beta$ -Tocopherol	0.52 $\pm$ 0.01 Ab	0.50 $\pm$ 0.01 Ab	0.50 $\pm$ 0.01 Ab	0.42 $\pm$ 0.01 Aa	n.d.	n.d.	0.884	***
$\gamma$ -Tocopherol	4.94 $\pm$ 0.14 Ad	4.92 $\pm$ 0.02 Ac,d	4.63 $\pm$ 0.15 Ac,d	2.70 $\pm$ 0.13 Ab	0.41 $\pm$ 0.10 Aa	0.33 $\pm$ 0.01 Aa	0.901	***
$\delta$ -Tocopherol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	–	–
Total tocopherols	13.09 $\pm$ 0.31 Ad	12.93 $\pm$ 0.02 Ad	10.78 $\pm$ 0.31 Ac	4.28 $\pm$ 0.29 Ab	0.80 $\pm$ 0.14 Aa	0.76 $\pm$ 0.01 Aa	0.844	***
<b>Soybean oil</b>								
$\alpha$ -Tocopherol	8.72 $\pm$ 0.31 Bb	8.61 $\pm$ 0.19 Bb	8.51 $\pm$ 0.27 Bb	8.55 $\pm$ 0.21 Bb	6.85 $\pm$ 0.26 Ba	7.20 $\pm$ 0.23 Ba	0.732	***
$\beta$ -Tocopherol	2.97 $\pm$ 0.16 Bc	3.01 $\pm$ 0.06 Bc	2.67 $\pm$ 0.08 Bb	2.58 $\pm$ 0.08 Ba,b	2.40 $\pm$ 0.05 a	2.38 $\pm$ 0.02 a	0.755	***
$\gamma$ -Tocopherol	62.92 $\pm$ 1.95 Bb	62.36 $\pm$ 1.35 Bb	64.02 $\pm$ 1.88 Bb	61.23 $\pm$ 1.24 Bb	51.16 $\pm$ 1.63 Ba	48.02 $\pm$ 1.82 Ba	0.867	***
$\delta$ -Tocopherol	33.01 $\pm$ 0.73 c	32.58 $\pm$ 0.63 c	32.84 $\pm$ 1.01 c	31.75 $\pm$ 0.64 b,c	29.93 $\pm$ 0.82 b	27.76 $\pm$ 0.71 a	0.872	***
Total tocopherols	107.62 $\pm$ 3.09 Bb	106.55 $\pm$ 2.20 Bb	108.04 $\pm$ 3.24 Bb	104.12 $\pm$ 2.15 Bb	90.34 $\pm$ 2.59 Ba	85.36 $\pm$ 2.51 Ba	0.882	***

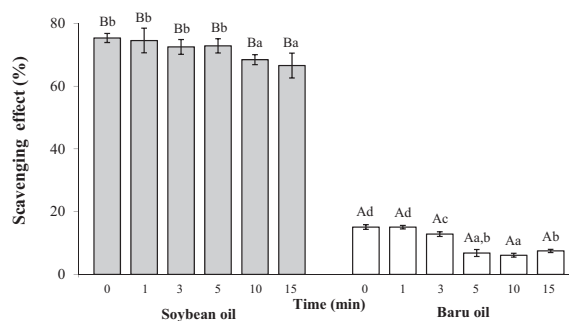
Means within a line, in each vegetable oil and in each parameter studied, with different letters differ significantly ( $P < 0.05$ ); among vegetable oils, for each microwave heating exposure time and parameter studied, mean values with different capital letters differ significantly ( $P < 0.05$ ).  $P > 0.05$ , n.s., not significant; \* $P \leq 0.05$ , significant correlation; \*\* $P \leq 0.01$ , very significant correlation; \*\*\* $P \leq 0.001$ , extremely significant correlation.

concerning their  $\gamma$ - and then  $\delta$ -tocopherol content, a difference that may contribute for a higher stability and antioxidant potential in soybean oil, as discussed in the next two sections.

### 3.5 Impact of microwave heating on antioxidant potential

The antioxidant capacity of baru and soybean crude oils exposed to different microwave heating exposure times was measured by the 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) scavenging assay. This method is an essential tool for estimating the antioxidant potential, more specifically, the antiradical activity of the oils studied. The results obtained are presented in Fig. 2. For authors' knowledge, this is the first report on the antioxidant activity of baru oil. Before heating, baru oil displayed a weak antiradical activity, about 15.1% while a considerable higher response was observed in soybean oils, reporting this oil the capacity to scavenge 75.4% of DPPH free radicals. When oils were exposed to microwave heating, baru oil reduce significantly its potential as soon as 3 min (12.8%). On the other hand, in soybean oils a significant loss of activity was only verified at  $t_{10\text{min}}$  (68.5%). After 15 min processing, baru oil retained only capacity to scavenge 6% of the DPPH ions while for soybean oil a 66.6% capacity was evaluated. Despite presenting similar degradation trends, soybean oil presented always higher antiradical capacity, and baru oil loss nearly 50% of its activity with microwave heating. On both oils, the antioxidant activity was extremely negatively correlated with the microwave heating exposure time, which means that with higher exposure time, the capacity to scavenge the free radicals of DPPH reduces ( $R^2 = 0.644$ ,  $P < 0.001$ , to baru oil and

$R^2 = 0.800$ ,  $P < 0.001$  for soybean oil). The higher antioxidant activity of soybean oil could be related with tocopherols content of this vegetable oil. Indeed, at  $t_{15\text{min}}$  soybean oil reported 100 times more tocopherols content than baru oil, which should have a direct influence in the results observed in antioxidant activity. The proportions of each tocopherol in both oils play a different role in the antioxidant activity displayed, and in our opinion,  $\gamma$ - and then  $\delta$ -tocopherol are preponderant in this case. Many works demonstrated the different capability of tocopherols to inhibit oxidation and to confer stability to vegetable oils, including soybean oil, corroborating our hypothesis [41–43]. In many cases natural antioxidants are added to vegetable oils to counteract thermal degradation [19, 44].



**Figure 2.** Scavenging effect of crude baru and soybean oils submitted to different microwave heating exposure times (mean  $\pm$  SD;  $n = 3$ ; bars within a same vegetable oil, with different minor letters differ significantly –  $P < 0.05$ ; among oils, bars at the same exposure time with different capital letters differ significantly –  $P < 0.05$ ).

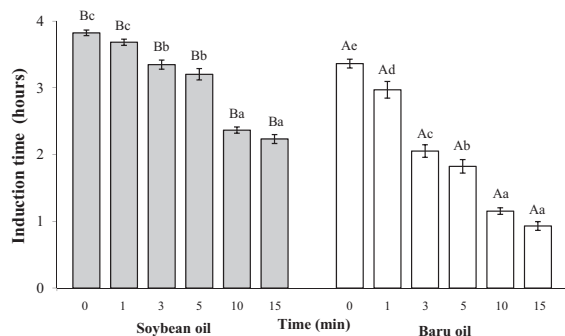


### 3.6 Microwave heating effect on oil oxidative stability

Measurement of oxidative stability of a vegetable oil is a routine parameter, giving an estimation of the resistance of the oil toward oxidation. Such estimation, however, depends on several factors, namely the conditions applied in the experiment (temperature and amounts of air bubbled), the degree of unsaturation of the oil, and presence of natural or added antioxidants in the oil. Unheated oils reported similar oxidative stabilities, reporting baru oil 3.4 h and soybean oil 3.8 h (Fig. 3). Just after 1 min of microwave processing, baru oil displayed a significantly lower resistance to oxidation ( $P < 0.001$ ), while in soybean oil the same was observed only at  $t_{3\text{min}}$  ( $P < 0.001$ ). Both oils oxidative stability was correlated with the increasing microwave heating exposure time: baru –  $R^2 = 0.853$  and  $P < 0.001$ ; soybean –  $R^2 = 0.945$  and  $P < 0.001$ . Furthermore, at  $t_{15\text{min}}$ , baru oil lost around 70% (0.9 h) of its initial stability, while soybean oil oxidative stability reduced around 40% (2.2 h), keeping 2.4 times more oxidative stability than baru oil.

In unheated oils, the baru oil fatty acids profile, with lower content of PUFA comparatively with soybean oil, confer it a similar stability to the soybean oil, with higher PUFA amounts and enough tocopherols to protect them efficiently against the aggressive pro-oxidant agents. However, with the constant increase of microwave heating exposure time, PUFA deteriorate easily in baru oil since there are few antioxidant molecules in oil composition, increasing oxidation reactions, therefore lowering the oxidative stability of the oil. This corroborates the high impact of microwave domestic use on oils stability, particularly baru oil.

The commercialization of baru oil advertises that it is an excellent source of antioxidant compounds with healthy properties to consumers. However, and being this the first report of this kind, the results obtained demonstrate that apart

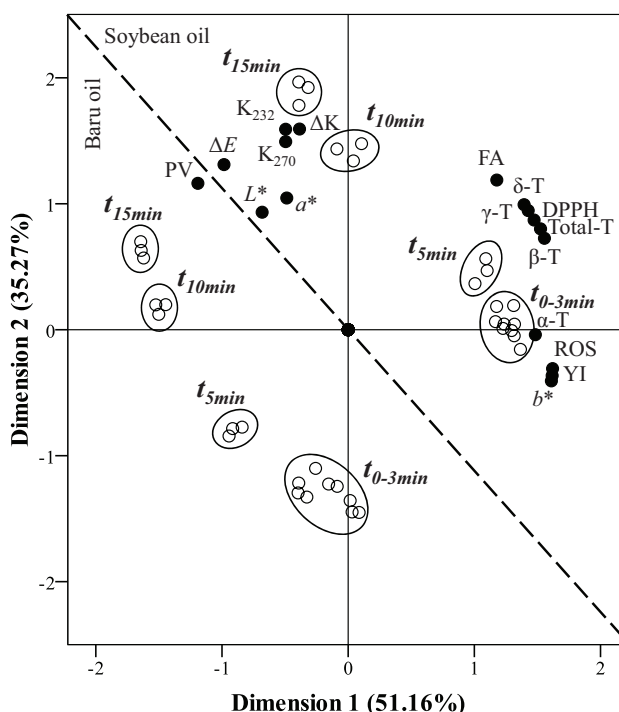


**Figure 3.** Oxidative stability of crude baru and soybean oil submitted to different microwave heating exposure times (mean  $\pm$  SD;  $n=3$ , bars within a same vegetable oil, with different minor letters differ significantly –  $P < 0.05$ ; among oils, bars at the same exposure time with different capital letters differ significantly –  $P < 0.05$ ).

from its equilibrated fatty acids profile, its antioxidant capacity is reduced, particularly when used for thermal processing.

### 3.7 Principal component analysis (PCA) of the obtained data

The information obtained in this work was applied in a PCA. The data used clearly allowed to distinguish the two types of oils, being represented in Fig. 4, and separated by a dashed line. With this PCA 86.43% of the total variance observed could be explained. The separation of the two oils is clear due to their different composition and different response to microwave heating. Soybean oils samples were mainly represented in the entire positive region of the second principal component. In each oil four groups were formed according to the exposure time applied. This information obtained from PCA indicates that similar heating times affect independently oils composition and properties. In both oils, samples unheated for 1 or 3 min were undistinguishable showing that microwave heating does not significantly degrade oils within such periods of exposure to microwave radiation. In fact soybean oils heated up to 5 min were grouped by PCA being mainly characterized by higher amounts of tocopherols, oxidative stability, and scavenging capacity of DPPH free radicals. The group  $t_{0-3\text{min}}$  from



**Figure 4.** Principal component analysis obtained from the quality parameters (FA, PV,  $K_{232}$ ,  $K_{270}$ , and  $\Delta E$ ), color ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $\Delta E$ , and YI), tocopherols content, antiradical capacity (DPPH), and oxidative stability (ROS). PCA factors explain 86.43% of the total variance.

soybean oil reported also a more intense yellow coloration, as observed by the values of  $b^*$  and YI (Table 1; Figs. 1 and 4). On the extreme opposite direction are represented samples from baru oil of  $t_{10\text{min}}$  and  $t_{15\text{min}}$  groups. Such disposition means that these groups were those who reported lower tocopherols content, lower antioxidant activity, lower stability and lower coloration, a direct consequence of the higher heating exposure applied. Both oils at  $t_{10-15\text{min}}$  reported high PV,  $\Delta E$ ,  $L^*$ ,  $a^*$ ,  $K_{232}$ ,  $K_{270}$ , and  $\Delta K$  values, all parameters that indicate degradation and oxidative status. We can retain that all data obtained is interconnected and that microwave heating influence oils in a distinct manner, being crude soybean oil more resistant and stable to microwave heating than baru oil.

Crude soybean oil was used as comparison, instead of refined, in order to eliminate confounding factors and evaluated them on the same basis. Still, based on the results obtained previously with refined soybean oil [19, 30], crude soybean oils performance under microwave heating was similar. Only free acidity,  $K_{270}$ , and *trans* content were different, a direct consequence of absence of refining in this work. Therefore, the previous conclusions on the deleterious effect of microwave on soybean oil still apply, but baru oil, despite its lower PUFA amounts, is even more vulnerable.

## 4 Conclusions

This study reflects the adverse effects of microwave heating on the composition and physical and chemical properties of baru and soybean crude oils. We concluded that microwave heating is more aggressive to baru oil than soybean oil. Until 3 min of heating, in general, no significant deterioration is observed in both oils. With higher heating periods, both oils become oxidized, with a higher degradation of PUFA and formation of oxidation compounds. Oil stability and antioxidant activity are directly affected by the exposure time, mainly due to the degradation of tocopherols. Oxidative stability of baru oil was reduced about 72%, antioxidant activity was reduced by an half, and tocopherol content decreased 94% with extensive microwave heating. Microwave heating causes bioactive and nutritional losses on both oils, and alter their appearance as well.

Based in the results obtained in this work, we can state that baru oil is very unstable when subjected to microwave heating. We recommend the use of this oil without heat or with minimal thermal processing possible in order to maintain its stability. Furthermore, more studies should be carried out to observe the behavior of baru oil under other different domestic culinary conditions, like baking, pan, and deep-frying.

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